Uranium and Technetium Bio-Immobilization in Intermediate-Scale Physical Models of an In Situ Bio-Barrier

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We investigated the long-term effects of ethanol addition on U and Tc mobility in groundwater flowing through intermediate-scale columns packed with uncontaminated sediments. The columns were operated above-ground at a contaminated field site to serve as physical models of an in situ bio-barrier for U and Tc removal from groundwater. Groundwater containing 4 μM U and 520 pM Tc was pumped through the columns for 20 months. One column received additions of ethanol to stimulate activity of indigenous microorganisms; a second column received no ethanol and served as a control. U(VI) and Tc(VII) removal was sustained for 20 months (∼189 pore volumes) in the stimulated column under sulfate- and Fe(III)-reducing conditions. Less apparent microbial activity and only minor removal of U(VI) and Tc(VII) were observed in the control. Sequential sediment extractions and XANES spectra confirmed that U(IV) was present in the stimulated column, although U(IV) was also detected in the control; extremely low concentrations precluded detection of Tc(IV) in any sample. These results provide additional evidence that bio-immobilization may be effective for removing U and Tc from groundwater. However, long-term effectiveness of bio-immobilization may be limited by hydraulic conductivity reductions or depletion of bioavailable Fe(III).

Introduction

Uranium and technetium are important groundwater contaminants at U.S. Department of Energy (DOE) waste sites because they are persistent, frequently mobile in aquifers, and toxic to potential receptors (*1*). Under oxidizing conditions, uranium exists as divalent uranyl ($U(VI)O_2^{2+}$), which forms soluble and mobile carbonate complexes in groundwater (*2*). Technetium is typically present as pertechnetate ($Tc(VII)O_4^-$), which is also highly soluble and mobile (*3*, *4*, *5*). The reduced oxidation states of uranium and technetium, U(IV) and Tc(IV), respectively, form sparingly soluble oxides (*5*). Thus, one way to remove U(VI) and Tc(VII) from groundwater is to stimulate the indigenous microbial community to create conditions that promote reductive precipitation of U(IV) and Tc(IV) in a process called bio-immobilization (*6*).

Bio-reductive precipitation of U(IV) and Tc(IV) can proceed either enzymatically or fortuitously under metal or sulfate reducing conditions (*7*, *8*). Bacteria including *Desulfovibrio desulfuricans* (*9*), *Geobacter metallireducens* (*10*), *Shewanella putrefaciens* CN32 (*11*), and *Desulfosporosinus* (*12*) are capable of enzymatic U(VI) reduction but microbial reduction can be complex and system-specific. For example, *Desulfovibrio desulfuricans* may couple U(VI) reduction to lactate or dihydrogen oxidation but only in the presence of sulfate (*9*, *13*). Tc(VII) may also be reduced enzymatically by *Desulfovibrio desulfuricans* (*7*, *14*, *15*) and *Deinococuss radiodurans* (*16*) or chemically by solids containing ferrous iron (*17*) or U(IV) (*18*).

Few field experiments have demonstrated that bio-immobilization of U and Tc can be achieved in situ. Senko et al. (*19*) conducted push−pull tests in an anaerobic landfill leachate-impacted aquifer using site groundwater amended with U. U(VI) reduction was observed concomitant with denitrification and sulfate reduction, although the contribution of microbial U(VI) reduction to observed U removal was not clear. Anderson et al. (*20*) conducted a natural gradient bio-immobilization study in a U contaminated aquifer. Initially, acetate additions resulted in Fe(III) reduction and decreased U concentrations. However, U(VI) concentrations eventually increased and aqueous Fe(II) concentrations decreased, which Anderson et al. (*20*) attributed to sulfate reducing bacteria out-competing Fe(III)- and U(VI)-reducers for acetate. Istok et al. (*21*) conducted push−pull tests at Oak Ridge National Laboratory using U and Tc contaminated groundwater. Ethanol additions stimulated denitrification and Fe(III), Mn(IV), U(VI), and Tc(VII) reduction in all tests.

These field studies have demonstrated the potential for bio-immobilization to remove radionuclides from groundwater but were also somewhat limited by test format or site selection. The Senko et al. and Istok et al. tests were short duration (<1200 h) and thus unable to provide information on longer-term changes in microbial activity or aquifer hydraulic conductivity. Although U, Tc, nitrate, and sulfate are common cocontaminants at many DOE waste sites, the Senko et al. study was conducted in aquifers without U, Tc, nitrate, or sulfate, and the Anderson et al. study was conducted without Tc or nitrate.

In this study, we investigated the long-term effects of electron donor addition on U and Tc mobility in groundwater flowing through above-ground, intermediate-scale columns deployed at a contaminated field site. We evaluated whether added ethanol could promote and sustain reductive precipitation of U(IV) and Tc(IV), thereby modeling a potential configuration for an in situ bio-barrier. The columns were packed with a mixture of uncontaminated sediments and continuously perfused with contaminated site groundwater containing U and Tc for 20 months. One column received ethanol additions to stimulate microbial activity and create anaerobic conditions favorable for bio-immobilization of U and Tc; a second column received no added ethanol.

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Materials and Methods

Materials and Apparatus. From 1951 to 1983, nuclear processing wastes leached from overlying storage ponds into the shallow, unconfined aquifer at Oak Ridge National Laboratory (*22*). The U.S. DOE established this site as a field research center (FRC) to provide researchers with access to contaminated sediment and groundwater. This study was designed to simulate in situ conditions in FRC area 2, where contaminated sediments forming the shallow (~4−6 m) aquifer consist of placed fill. The fill is comprised of a mixture of shale saprolite and gravel mined from nearby outcrops of the Maynardville Limestone (*23*). Uncontaminated fill was not available and so experiments were conducted with a constructed fill prepared by combining uncontaminated saprolite from the FRC background site (background sediment) (~11 wt %) with Maynardsville Limestone (89 wt %) that had been crushed and sieved (<0.6 cm). See Supporting Information for sediment characteristics. The sediment mixture was packed in layers into identical columns made from horizontal 15.2 cm diameter by 243.8 cm long polyvinyl chloride pipe. A small quantity of sodium bicarbonate (0.3 wt %) was added to the sediments during packing to minimize U(VI) sorption prior to stimulating reducing conditions, as was previously done in our laboratory studies (data not shown). Subsequent porewater measurements of Na^+ indicated that the added sodium bicarbonate was flushed from the column during the first five weeks of operation. The columns were equipped with an inlet, outlet, eight sampling ports, manometers and four ethanol injection ports distributed along the column length (Supporting Information, Figure 1). The columns were deployed in a climate controlled trailer above the contaminated aquifer at the FRC in Oak Ridge, TN. Contaminated site groundwater from a nearby well (well GW835, (*24*)) was pumped through both columns using piston pumps. This groundwater was aerobic (dissolved oxygen ~6 mg/L), with pH 6.4, and nitrate (1 mM), sulfate (1 mM), U (4 μM) and Tc (520 pM) concentrations typical of FRC area 2 groundwater. Pumping rates were selected to yield porewater velocities similar to those reported for site groundwater (0.5−2 m/day, (*24*)). Pumping rates were monitored weekly, but varied during the long experiment duration at this remote site; average porewater velocities were 0.61 and 1.4 m/day for the stimulated and control columns, respectively.

A syringe pump was used to add 0.6 mL of ethanol (190 proof ethyl alcohol) daily to the inlet and each injection port of the stimulated column; the control received no added ethanol. Ethanol was used for its convenience for automated injection and because concentrated ethanol inhibited microbial growth in injection tubing and ports. Water samples (15 mL) were collected from all sampling ports twice per week for the first 3 months and once per week thereafter. Inorganic anions, pH, ethanol, U, and Tc were analyzed in all collected pore water samples. A single set of zero headspace pore water samples were collected for volatile fatty acid and methane analysis after 40 pore volumes passed through the stimulated column. Sediment samples were collected after ~79 and 72 pore volumes had passed through the stimulated and control columns, respectively (Experimental Timeline, Figure 2, Supporting Information). Brief descriptions of analytical methods are given below; see Supporting Information for details.

Characterization of Sediment Mixture. Sediment mineralogy was characterized using chemical extraction, X-ray diffraction and X-ray fluorescence methods. Total Fe(III) was calculated as the dithionite−citrate−bicarbonate extractable Fe less the weak acid extractable Fe(II). The limestone was completely dissolved in weak acid; resulting Fe(II) was assumed to be structural and was neglected in the Fe(III) calculation (Table 1, Supporting Information).

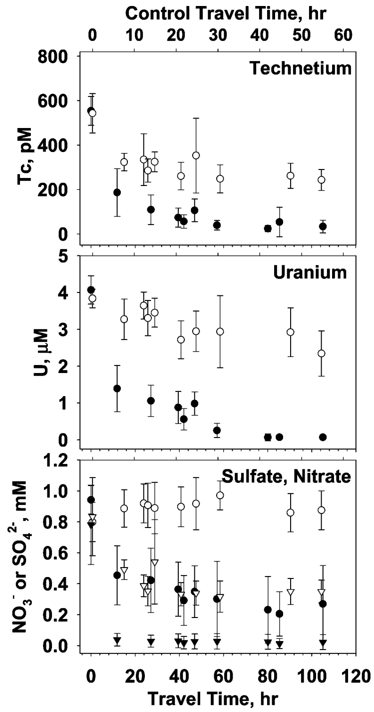

FIGURE 1. Average concentration profiles for the ethanol stimulated column (solid symbols, 20 months) and control (open symbols, 16 months) for the entire experiment. Inlet concentrations correspond to time zero and error bars represent one standard deviation. Triangles represent nitrate concentrations; circles represent all other analytes.

Porewater Analyses. pH was measured using a glass electrode. Inorganic anion (NO_3^-, SO_4^{2-}, Br^-, NO_2^-) and organic anion (acetate, propionate, and butyrate) concentrations were measured by ion chromatography. Ethanol and methane were measured using gas chromatography. Uranium concentrations were measured using kinetic phosphorescence analysis. Technetium was measured by liquid scintillation counting.

Sediment Analyses. Sediment samples were collected by temporarily removing sampling port fittings under an Ar atmosphere. Sediment samples were sequentially extracted to quantify U using previously published methods (*25*, *26*). Briefly, sediments were extracted first with a known mass of deoxygenated DI water; next with deoxygenated, 1 M HCO_3^-; and finally were incubated with concentrated nitric acid for ~24 h. Using previously published criteria (*25*), uranium extracted from the sediment by rinsing with DI water and HCO_3^- was assumed to be U(VI), and acid extractable uranium (after DI and HCO_3^- rinses) was assumed to be U(IV). The presence of U(IV) in selected samples was verified using X-ray absorption near edge spectra (XANES) at MR-CAT 10ID beamline (*27*). Fe(II) and Fe(III) content in selected sediment samples were determined using Mössbauer spectra collected at 4, 77, or 298 K (*28*). Spectra were fitted using Recoil version 1.0 (K. Lagarec and D. Rancourt, Department of Physics, University of Ottawa)

Data Analysis. Hydraulic conductivity was computed from measured pumping rates and hydraulic heads. Tracer tests were conducted by amending influent groundwater with 100 mg/L Br^- and sampling to obtain breakthrough curves. Porewater velocities were determined by fitting Br^- breakthrough concentrations to the advection dispersion equation using CXTFIT (*29*). Porewater velocities were used to calculate travel times from the inlet to each sampling port. Apparent zero-order removal rates for nitrate, sulfate, U, and Tc were computed by dividing measured changes in concentration

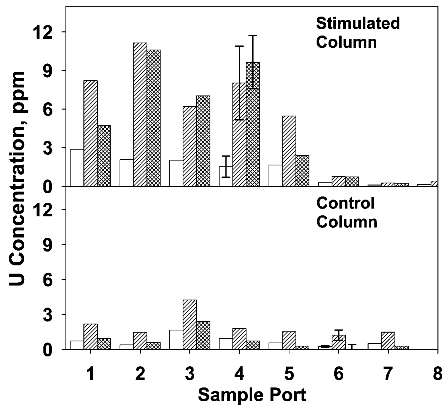

FIGURE 2. Bars represent mass of U extracted per dry mass of sediment. The first bar represents DI extractable U, the second represents HCO_3^- extractable U and the third bar represents HNO_3 extractable U. Error bars represent standard deviation of triplicate analysis, which was done for two sediment samples only.

between adjacent sampling ports by the travel time. Aqueous mass balance calculations were performed by integrating measured pumping rates and differences in inlet and outlet concentrations.

Geochemical Modeling. Activities of aqueous and solid U and Tc species were computed as a function of pH and redox potential using the Geochemist's Workbench v. 6.0 (*30*). The thermodynamic database was edited to include $CaUO_2(CO_3)_3^{2-}$ and $Ca_2UO_2(CO_3)_3$ complexes using data in Berhard et al. (*31*). Measured sulfate reduction presumably governed redox potential in the stimulated column sediments. The Nernst equation was used to calculate the associated range of redox conditions in the stimulated column sediments (see Supporting Information Table 5 for calculations). Partial pressure of CO_2 in column sediments was calculated by assuming all ethanol was mineralized to CO_2; this assumption is conservative by providing maximum available carbonate in solution for uranium complexation.

Results and Discussion

Nitrate, sulfate, U, and Tc concentrations initially decreased in both columns during the first two weeks of operation. In the stimulated column, this trend continued and nitrate, sulfate, U, and Tc continued to decrease along the column length for the remainder of the experiment. After two weeks U and nitrate concentrations in the stimulated column effluent were essentially zero. In the control column, sulfate and U concentrations increased after two weeks and eventually were nearly constant along the column length, while nitrate and Tc concentrations continued to decrease, but to a lesser extent than in the stimulated column. Differences between the stimulated and control columns are summarized in Figure 1, which shows the long-term average concentrations and confidence intervals for both columns during the entire experiment. Due to differences in pumping rates, porewater velocities were smaller and computed travel times were larger in the stimulated column than in the control; travel times ranged from 0 (inlet) to 105 h (outlet) in the stimulated column and from 0 (inlet) to 55 h (outlet) in the control. In the stimulated column, average nitrate concentration decreased from 0.8 to ~0 mM and sulfate decreased from 1 to ~0.2 mM. The average U concentration decreased from 4 to ~0 μM and Tc decreased from ~600 to ~20 pM. In the control, concentrations decreased much less to ~0.4 mM (nitrate), ~0.9 mM (sulfate), ~3 μM (U), and 275 pM (Tc) (Figure 1). Measured nitrate, sulfate, U, and Tc concentrations at all sampling ports were significantly smaller in the stimulated column than in the control ($p < 0.01$). Small quantities of ethanol delivered to the control by contaminated drums used to store site groundwater, or oxidation of sediment organic matter, likely stimulated limited microbial activity in the control column and may be responsible for some of the observed decreases in analyte concentrations. Sorption was likely responsible for some decrease in U concentrations in both the stimulated and control columns.

Computed removal rates were significantly larger in the stimulated column than in the control; the largest rates were observed in sampling port 1 of the stimulated column and were ~0.06 mM/hr (nitrate), 0.05 mM/hr (sulfate), ~0.4 μM/hr (U), and ~40 pM/hr (Tc) (Table 2, Supporting Information). Porewater sample collection and analysis continued until early June, 2005 when the experiment was terminated. Concentration profiles and removal rates remained consistent with those reported here and confirmed that ethanol additions to the stimulated column supported the simultaneous removal of U and Tc from site groundwater for the duration of the experiment.

Mass balance calculations based on porewater and flow rate data indicated that significantly more U and Tc were deposited (i.e., removed from groundwater) in the stimulated column than in the control (Table 3, Supporting Information). Stimulated column sediments contained more U and a higher proportion of acid extractable U than control sediments (Figure 2). When U sediment data were normalized to account for differences in groundwater volumes pumped through

TABLE 1. Results of Mass Balance Performed on U and Tc[a]

	stimulated column	control column
cumulative U extracted from sediment, μg	110	30.0
cumulative dry sediment mass extracted from all sample ports, g	10.6	8.73
average extractable sediment U concentration, ppm	10.4	3.44
volume of groundwater passed through columns at time of sediment collection, L	809	252
average extractable sediment U concentration normalized to total water volume, ppm	***10.4***	***11.0***
aqueous mass balance derived sediment U concentration normalized to total water volume, ppm (for comparison purposes)	7.1	5.1
total acid extractable U operationally	44.2	6.50
average acid extractable U concentration, ppm	4.17	0.74
average acid extractable concentration normalized to total water volume, ppm	***4.17***	***2.39***

[a] Predicted control values were scaled to account for less total groundwater volume passing through this column at the time sediment samples were collected for U extraction.

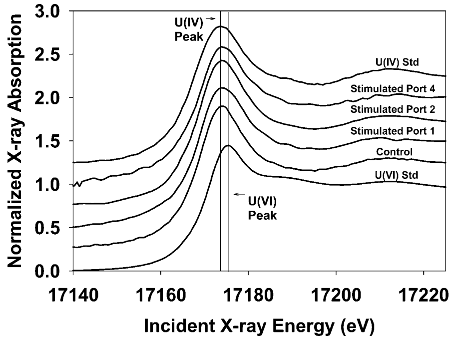

FIGURE 3. XANES spectra (U L_{III}-edge normalized absorption) spectra from the stimulated and control sediment samples, U(VI) standard, and U(IV) standard.

each column, the total U content of stimulated and control sediments were equal but the stimulated sediment contained 43% more acid extractable U than the control (Table 1). Extremely small Tc concentrations precluded sediment Tc analyses.

The position of the adsorption edge and the shape of the oscillatory portion of the XANES spectra, which closely resembled the U(IV) standard, confirmed the presence of mostly U(IV) (>85%) in stimulated sediments (Figure 3). The proportion of U(IV) in samples analyzed by XANES was substantially larger than the proportion of acid extractable U in the same samples (Figure 2, Supporting Information Table 4) and is attributed to differences in sediment size fractions analyzed by the two methods, small-scale mineralogical heterogeneities, and the likelihood that acid extractable U may contain a mixture of U(IV) and U(VI). An average sediment volume of ~0.9 cm^3 was extracted for U analysis per sediment sample, which is much larger than the volume (~0.01 cm^3) interrogated by XANES. It was only possible to analyze one control sample. The control sample with the most reduced appearance was selected for this purpose and it too contained mostly U(IV) (Figure 3). The presence of U(IV) in the control was unexpected but is likely due limited microbial activity supported by sediment organic matter oxidation (which is consistent with long-term average concentration profiles that showed decreased nitrate and technetium concentrations in the control column). Mössbauer spectra indicated that stimulated sediments contained higher levels of Fe(II) and lower levels of Fe(III) compared to the pristine sediment mixture used to pack the columns, indicating that ethanol additions stimulated Fe(III)-reducing microbial activity (Figure 4). In addition, Mössbauer spectral analysis confirmed FeS_x presence in the stimulated sediment.

Wan et al. (*33*) and others showed that the soluble complex, $Ca_2UO_2(CO_3)_3$, may decrease the stability of uraninite (UO_2) in the presence of calcite (*34*), suggesting that a portion of the observed aqueous U loss in the stimulated column may have been due to U(VI) sorption or precipitation of U(VI) bearing mineral phases. However, geochemical modeling indicated that amorphous U(IV)O_2 was the most stable mineral phase under sulfate and Fe(III)-reducing conditions in the stimulated column, with or without the inclusion of U−Ca complexes in the thermodynamic database (Figure 5). Pertechnetate (Tc(VII)O_4^-) sorbs only minimally to these sediments and may be reduced microbially or by reaction with Fe(II) or sulfide (*17*, *35*, *36*); therefore, it is likely that decreased Tc concentrations in the stimulated sediments were the result of Tc(VII) reduction. Geochemical modeling indicated that Tc_2S_7 was the most stable mineral phase under sulfate and Fe(III)-reducing conditions in the stimulated column (Figure 5). A recent study by Lukens et al. (*37*) provided spectroscopic evidence that suggested Tc_2S_7(s) is actually a reduced Tc(IV)-sulfide with stoichiometry $Tc_3S_2(S_2)_4$(s) or Tc_3S_{10}.

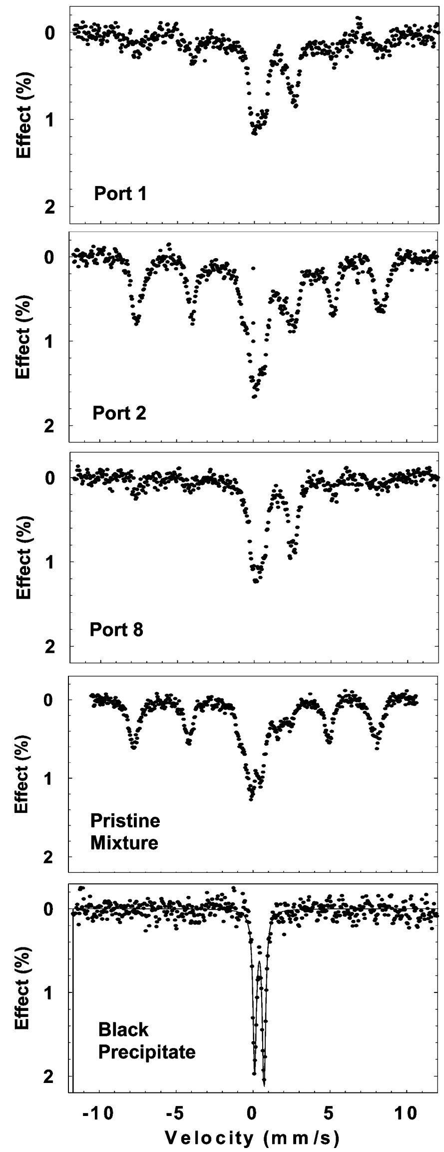

FIGURE 4. Mössbauer spectra at 4K for three stimulated sediment samples, for the pristine sediment mixture used to pack the columns, and for a black precipitate present in the stimulated sediments samples. Port 2 and the pristine mixture exhibited the highest Fe(III)-oxide signals, as evidenced by the prominent spectral sextet. Ports 1 and 8 exhibited increased Fe(II) signals, as evidenced by increased absorption at ~2.5 mm/s. The solid line in the precipitate spectrum represents the *Recoil* fitted pattern, which was consistent with an iron sulfide mineral phase.

Anderson et al. (*20*) suggested that Fe(III) availability is an important factor for the long-term effectiveness of U bioimmobilization and that if bioavailable Fe(III) is exhausted, or becomes otherwise unavailable, the biogeochemical system may shift in such a way as to preclude U(VI) reduction. The pristine sediment used to pack the stimulated column initially contained ~2.2 mol of Fe(III). Based on increased sediment Fe(II) indicated by Mössbauer spectra, sustained sulfate reduction, and detection of methane in the porewater samples (data not shown), it is likely that a substantial fraction of the initially bioavailable Fe(III) was reduced.

Hydraulic conductivity of the sediment pack in the stimulated column was ~1.6×10^{-4} m/sec initially but decreased by approximately 1 order of magnitude to ~2.5×10^{-5} m/sec by the end of the experiment. Precipitation of reduced metals and assumed increased biomass likely contributed to the observed decrease in hydraulic conduc-

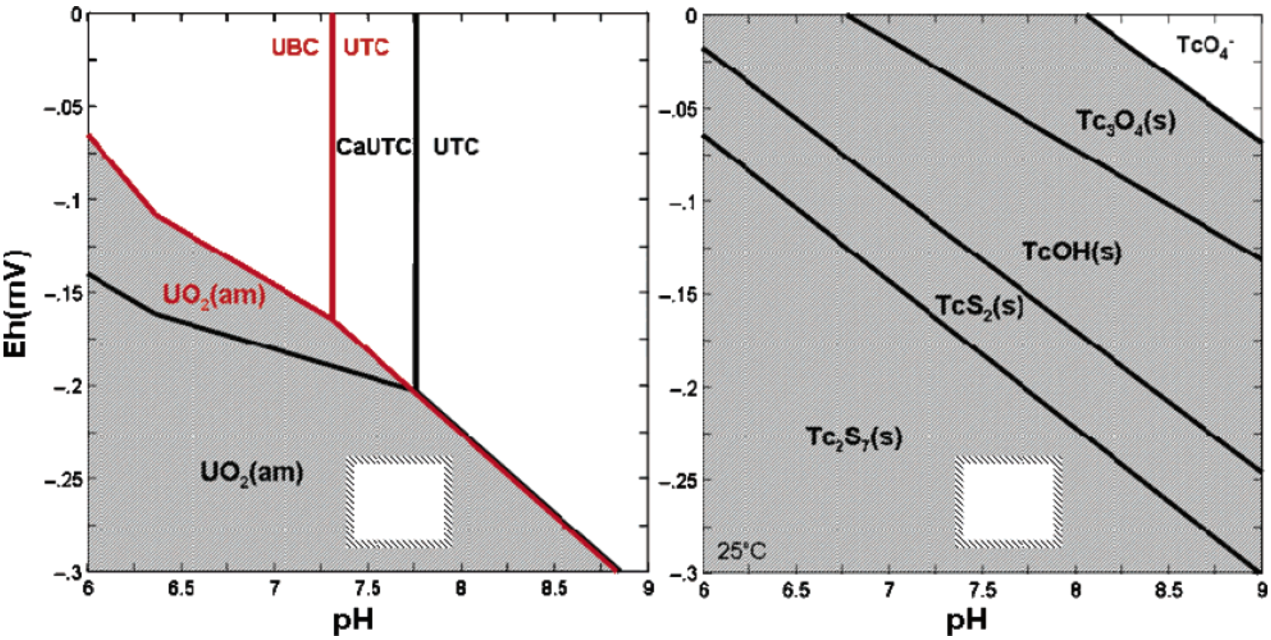

FIGURE 5. Chemical speciation of 1 M uranium (left panel) and 1000 pM technetium (right panel) with sulfate equal to 1 mM and pCO_2 equal to 0.025 atm, which was calculated by assuming all injected ethanol was mineralized to CO_2 at average pH 7.6. Black lines and red lines represent aqueous U speciation with and without calcite, respectively (left panel). Filled areas represent solid phases and inset squares indicate representative stimulated column redox conditions (calculations in Supporting Information). UBC, UTC, and CaUTC represent $UO_2(CO_3)_2^{2-}$, $UO_2(CO_3)_3^{3-}$, and Ca $UO_2(CO_3)_2$. All activity coefficients were set equal to 1.

tivity. Measured hydraulic conductivities of the stimulated sediment remained within the wide range of published conductivity values for the FRC aquifer (1.0×10^{-4} to 1.0×10^{-11} m/sec (*38*, *39*)) but clearly, even longer-term and, perhaps, in situ experiments are needed to predict the magnitude of hydraulic conductivity decrease that could occur if bioimmobilization is used to treat U and Tc contaminated groundwater at the FRC. Hydraulic conductivity of the sediment pack in the control was initially 0.004 cm/sec but increased to ~0.03 cm/sec; the explanation for the increase is unknown.

Two other long-term laboratory experiments have been conducted to study bio-immobilization of U in packed sediment systems. Gu et al. (*32*) conducted a small scale (~15 cm) column experiment for ~8 months by recirculating artificial groundwater amended with ethanol and sulfate through U-contaminated FRC sediment. Aqueous U concentrations were reduced from ~71 to ~1.3 μM after 140 days but increased to ~3.4 μM by day 245 under methanogenic conditions. Wan et al. (*33*) conducted a small scale (~20 cm) column experiment for ~ 16 months by continuously pumping lactate amended artificial groundwater through U-contaminated FRC sediment. Aqueous U concentrations were reduced from ~20 to 0.03 μM by day 60 but subsequently increased to ~1 μM for the duration of the study under methanogenic conditions.

Our study complements these two studies. To our knowledge, this study is first to demonstrate long-term removal of U and Tc from contaminated groundwater by stimulating microbial activity in an (initially) uncontaminated sediment system. However, additional research is needed to quantify the effects of hydraulic conductivity reduction and depletion of bioavailable Fe(III) on the long-term effectiveness of biostimulation for treating U- and Tc-contaminated groundwater.

Acknowledgments

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Note Added after ASAP Publication

Due to a production error, a mistake appeared in the Results and Discussion section in the version posted ASAP October 7, 2006; the corrected version was published ASAP October 10, 2006.

Supporting Information Available

A schematic of the columns, detailed descriptions of the analytical methods and supplementary results. This material is available free of charge via the Internet at http://pubs.acs.org.

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